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Research Article

Effect of Vitamin E as an Adjuvant to Metronidazole in Experimental Model of Peritonitis

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Abstract

Introduction: Peritonitis is the major surgical burden, even after use of higher broad spectrum antibiotics the mortality is high. Vitamin E is an antioxidant; which inhibits of lipid peroxidation, reduces and neutralizes free radicals; [the free radical scavenger effect.] This study assesses the effect of intra-peritoneal Vitamin E and Metronidazole instillation, either singly or in combination, in experimentally produced faecal peritonitis model.

Materials and method: This study was conducted in NSCB Government Medical College, India, on 84 healthy Albino rats of weight(100 -150 grams).All rats were undergone laparotomy with cecal ligation and puncture of different sizes to produce different grades of peritonitis and then divided into four groups according to solution instilled in their peritoneal cavity. 1. Group C (normal saline), 2. Group E (vit E), 3.Group M (metrogyl) 4. Group EM (vit E+ metrogyl) All rats were re-explored on 5th day and assessed for pus pockets, and adhesion.

Results: Metronidazole & Vitamin E group (ME) showed significant decrease in pus pocket formation as compared to control group in all its subgroups(subgroup I-p>0.018, subgroup II p>0.011) .

Conclusion: The rat peritonitis model of caecal ligation and puncture is satisfactory and very useful for study. In our study the antibiotic and antioxidant combination is superior to control group or antibiotic or antioxidant group when used alone; in prevention pus pocket formation.

Keywords: **:** Peritonitis; Experimental model; Metronidazole; Vitamin E

1. Introduction

Vitamin E is one of the antioxidant used widely. It inhibits of lipid peroxidation, reduces and neutralizes free radicals, this is what we call, 'the free radical scavenger effect'.[1,2] The disastrous effect of free oxygen radicals is well established now; it is very well known fact that the peroxidation of plasma membranes and cell takes place due to the adversity of action of free radicals.[3]

The mortality is very high in cases of peritonitis; these cases are major burden in surgical wards, even after due care and use of higher broad spectrum antibiotics the mortality is high.[4,5] The organ dysfunction which is caused by peritonitis is worsened by free oxygen radicals those are the result of endotoxic shock.[6,7,8] The Caecal Ligation and Puncture (CLP) technique of peritonitis model in albino rats is good, economical and easily reproducible in experimental model for evaluation of new treatment modality.[9]

The present study was carried out to assess the effect of intra-peritoneal Vitamin E and Metronidazole either singly or in combination, in experimentally produced faecal peritonitis model.

2. Materials and Method

This study was conducted in Netaji Subhash Chandra Bose Government Medical College, India from March 2004 to October 2004.The study was done on 84 healthy Albino male and female rats of weight between 100 grams to 150 grams, to see the effect of Vitamin E in experimentally induced peritonitis on pus and adhesion formation. The due approval and clearance from Institutional Ethics Committee for animals was obtained. According to the guidelines of Institutional Ethics Committee the animals were treated in humanely manner. After proper labelling of rats they were kept in hygienic conditions in separate cages.

2.1 Materials used: *Mus Norvagicus* Albinus Rats, 1ml Syringe, 2ml Syringes, sterile needles, silk 3-0, Povidone Iodine Solution, Ether, Vitamin E, metronidazole solution, distill water and Normal saline for injection and surgical tray and materials for laparotomy of rats.

2.2 Procedure: All rats kept in humanely manner in separate cages, their diet consisted of red Bengal gram and water. The study groups were kept at room temperature throughout the experimental period. Ether was used as an inhalational anesthetic agent by. The rats were kept nil by mouth for 12 hours before the first laparotomy.

Under all aseptic precaution, lower abdomen of rat was opened in midline; the caecum was identified and withdrawn. It was ligated beyond the ileo-cecal junction with silk 3-0 while maintaining the intestinal patency. Puncture of different sizes were made in caecum and then by applying pressure at ligated part of caecum, faecal matter extruded out followed by constant leakage of faecal matter into the peritoneal cavity. The subgroups in each group of different sizes punctures were as follows: 1. Subgroup I - Caecum was punctured with 22-gauge needle.2. Subgroup II - Caecum was punctured with 18-gauge needle.3. Subgroup III - Caecum was punctured with surgical blade no. 15; 5 mm linear incision was given.

Group C (Control group) - In this group laparotomy was done. In each rat, caecal ligation and puncture was done. According to different size of puncture, all rats were subdivided into 3 subgroups. Abdomen was closed after instilling 1 ml normal saline/100 gm of body weight intra-peritoneally.

Group E (vitamin E group) - In this group laparotomy was done. In each rat, caecal ligation and puncture was done. According to different size of puncture all rats were subdivided into 3 subgroups. Abdomen was closed after instilling vitamin E intra-peritoneally 100 mg /100 gm of body weight.

Group M (Metronidazole group) - In this group laparotomy was done. In each rat caecal ligation and puncture was done. According to different size of puncture all rats were subdivided into 3 subgroups. Abdomen was closed after instilling Metronidazole 5 mg intra-peritoneally /100 gm of body weight.

Group ME (Metronidazole &Vitamin E group) - In this study group laparotomy was done. In each rat, caecal ligation and puncture was done. According to different size of puncture all rats were subdivided into 3 subgroups. Abdomen was closed after instilling vitamin E 100 mg and metronidazole 5 mg intra-peritoneally /100 gm of body weight.

Abdomen was closed in two layers in all of the above subgroups. Each group as kept in different cage and labeled accordingly. Each rat received 5 ml of normal saline/100 gm of body weight subcutaneously as fluid resuscitation. Each rat was given antibiotic intramuscularly through insulin syringe as follows:

Name of Antibiotic	Site	Dose
Ceftriaxone	Right thigh	50mg/kg/day as a single dose
Amikacin	Right forearm	20mg/kg/day in two divided doses
Metronidazole	Left thigh	20mg/kg/day in three divided doses

Rats were again kept nil by mouth for 12 hours and then free access to water and Bengal gram was allowed. Rats that died were excluded from the study. The rats were sacrificed on the day 5; each subject was assessed on the basis of; pus pocket, pus over caecum and adhesions.

3. Observation and Results

Following observations were noticed. Laparotomy was done on day 0 and re-exploration was done on fifth day. The animals, those died, were excluded from the study. Laparotomy was done through the lower midline incision under ether anesthesia. Animals were subjected to sacrifice on 5^{th} day; pus over caecum, adhesion and pus pocket were observed on 5^{th} day. The detail of pus over caecum, pus pocket and adhesion was recorded and it was follows:

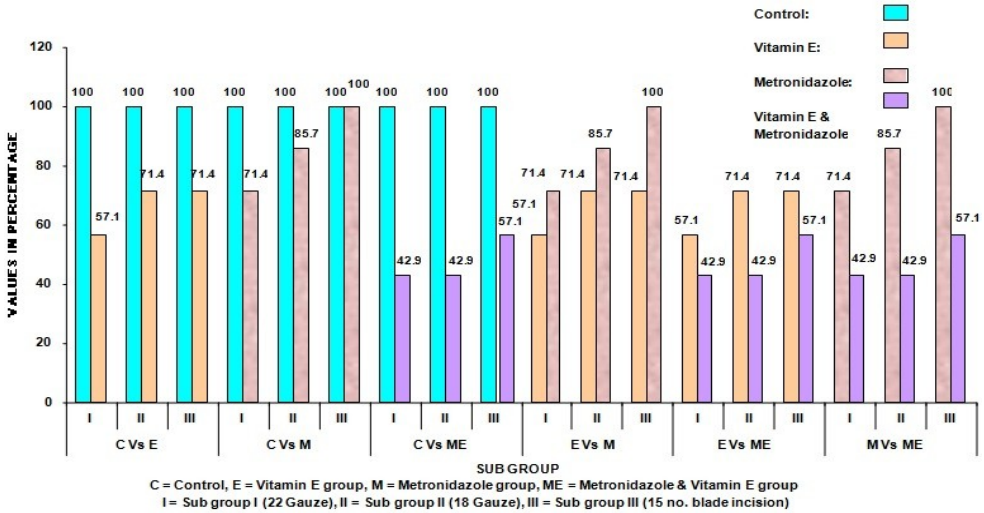
Table No. 1. Comparative results of all groups

Group	Sub group	PP number of rats (percentage)			PC number of rats (percentage)		
		C	E	P	C	E	P
C Vs E	I	7(100%)	4(57.1%)	0.051	6(85.7%)	3(42.9%)	0.094
	II	7(100%)	5(71.4%)	0.127	6(85.7%)	4(57.1%)	0.237
	III	7(100%)	5(71.4%)	0.127	7(100%)	6(85.7%)	0.299
C Vs M		C	M	P	C	M	P
	I	7(100%)	5(71.4%)	0.127	6(85.7%)	5(71.4%)	0.515
	II	7(100%)	6(85.7%)	0.299	6(85.7%)	4(57.1%)	0.237
	III	7(100%)	7(100%)	1	7(100%)	5(71.4%)	0.127
C Vs ME		C	ME	P	C	ME	P
	I	7(100%)	3(42.9%)	0.018	6(85.7%)	3(28.6%)	0.031
	II	7(100%)	3(42.9%)	0.018	6(85.7%)	3(42.9%)	0.094
	III	7(100%)	4(57.1%)	0.051	7(100%)	4(57.1%)	0.351
E Vs M		E	M	P	E	M	P
	I	4(57.1%)	5(71.4%)	0.577	3(42.9%)	5(71.4%)	0.280
	II	5(71.4%)	6(85.7%)	0.515	4(57.1%)	4(57.1%)	1
	III	5(71.4%)	7(100%)	0.127	6(85.7%)	5(71.4%)	0.515
E Vs ME		E	ME	P	E	ME	P
	I	4(57.1%)	3(42.9%)	0.593	3(42.9%)	3(28.6%)	0.577
	II	5(71.4%)	3(42.9%)	0.280	4(57.1%)	3(42.9%)	0.593
	III	5(71.4%)	4(57.1%)	0.577	6(85.7%)	4(57.1%)	0.237
M Vs ME		M	ME	P	M	ME	P
	I	5(71.4%)	3(42.9%)	0.280	5(71.4%)	3(28.6%)	0.109
	II	6(85.7%)	3(42.9%)	0.094	4(57.1%)	3(42.9%)	0.593
	III	7(100%)	4(57.1%)	0.105	5(71.4%)	4(57.1%)	0.577

(C= Control, E = Vitamin E, M = Metronidazole, ME= Metronidazole + Vitamin E)

I = Sub group I (22 gauze needle), II = Sub group (18 gauze needle), III = Sub group III (Linear puncture with 15 number blade); PP = Pus pocket; PC=Pus over caecum; P=P value

Figure-1- Graph showing distribution of pus pocket in various group & subgroups



Analysis of Table - 1

i. Control group (C) vs. Vitamin E group (E) – No result was statistically significant however the percentage value of group E were superior to group C in all parameters under study i.e. pus pocket formation and pus over caecum.

ii. Control group (C) vs. Metronidazole (M) group – No result was statistically significant however the percentage value of group M were superior to group C in all parameters under study. It shows that pus pocket formation is lower with metrogyl.

iii. Control group (C) vs. Metronidazole & Vitamin E group (ME)– Following results are statistically significant. Subgroup I in which puncture size were small; metrogyl and vitamin combination produce significant decrease in pus pocket formation (P-0.018). Even with large puncture size(Subgroup II, which produces more severe peritonitis; metrogyl and vitamin combination again produce significant decrease in pus pocket formation (P-0.011) In all other parameter, the results of Metronidazole & Vitamin E are better in terms of percentages although not significant.

iv. Vitamin E group (E) vs. Metronidazole group (M) – The results of Vitamin E are largely superior to Metronidazole however not statically significant.

It is evident from the study done that all the study groups (i.e., Vitamin E, Metronidazole and Metronidazole & Vitamin E) are superior to control group (Normal Saline) in prevention of the effects of peritonitis i.e. pus pocket formation and pus over caecum decreased with instillation of vitamin E and/or Metronidazole. The trend is also maintained within the subgroups (I, II and III) with striking regularity. Increase in size of caecal puncture also increases pus pocket, pus over caecum and culture positivity. It is also apparent that the Metronidazole & Vitamin E group is by far the most superior group and even at times when the results are not statistically significant; it maintains its superiority in terms of absolute percentages. The study underlines the superiority of Metronidazole & Vitamin E group with respect to all the other 3 groups under investigation. However, amongst the Vitamin E group and Metronidazole group the results are strikingly close and invariably head towards a nail biting finish. Though the group Vitamin E retains slight edge over group Metronidazole the difference between the two is miniscule. In certain parameters results of Metronidazole are better which probably denote that the results are too close for comparison.

Within the subgroups, the trend is again uniform. The subgroup I show the best results followed by II and then III in all the parameters under study. However, the trend signified amongst the study group is also maintained. Metronidazole & Vitamin E is better than Vitamin E, which in turn is better than Metronidazole. All these groups are superior to the control group.

The result of 22 gauze needle is better than 18-gauze which in turn is better than linear puncture by no 15 blade. Adhesions were present in 100% of subjects and hence it was not used as a part of calculations.

4. Discussion

Peritonitis has been one of scariest problem the in surgical units from long time. Surface area of peritoneum is large, the total surface area, it is 3 times that of the skin. The mortality is as high as 10% in cases of peritonitis. The huge morbidity and mortality has propelled authors to undertake the study for research.

Many agents were used intra-peritoneally for prevention of peritonitis. The oxygen free radicals play utmost important role in peritonitis and its further progress.

Our present study was aimed at assessing the role of antibiotic, free radical scavenger and their combination in prevention and limiting the effect of peritonitis. We also tried to assess the effect of size of perforation on the overall outcome of peritonitis. Efforts were made to remove bias and confounding factors and to systematically qualify and quantify the results obtained.

Caecal ligation and puncture was used as a method of producing peritonitis. This method was also studied earlier and found to produce invariably satisfactory peritonitis. It was found that the severity of sepsis obtained with caecal ligation and puncture can be enhanced by varying the size of puncture. In present study, similar results were found as pus pocket formation and pus over caecum were more in rats who had larger caecal perforation. Caecal perforation size also seems to influence the systemic toxemia produced in peritonitis as proved by E.[8]

The subjects were re-explored on the fifth day. This allowed enough time for the rats to come out of immediate postoperative stress and helped us to observe the short term results of the experimental drugs. Adhesions were found in 100% of subjects irrespective of group, subgroup. The trauma caused by caecal ligation and puncture has been the most important factor.

The presence of adhesion was thus a constant factor in the study done.[9] Pus pockets were present in all the rats of the control group for understandable reason, irrespective of size of perforation. Vitamin E, Metronidazole and

Metronidazole &Vitamin E gave better results when compared with 'Control'. The 'Metronidazole & Vitamin E' group showed least number of Pus Pockets. While amongst Vitamin E and Metronidazole group alone, the results of Vitamin E group were marginally better than Metronidazole group. While the combination of antibiotic and antioxidant (Metronidazole &Vitamin E) showed significant improvement over the Control group, probably the local free radical scavenger action provided Vitamin E with the slight edge over Metronidazole in prevention of pus pocket. Amongst the subgroups as expected, the size of perforation increase, the Pus Pocket becomes more prevalent. Localized abscess near caecal perforation site is similar to the pus pockets of the present study detected in control group.[10]

Pus over caecum also followed the same trend as the Pus Pocket in terms of groups but the line of demarcation between Vitamin E group and Metronidazole group thinned out further and the trend was not only variable but also even reverse at times. On fifth day, Vitamin E regained its lead possibly because of a better antioxidant effect.[11,12,13]

As the size of perforation increases the presence of local pus showed marked increase. The long term effect of drugs used seems to be a more plausible reason for the effect observed. However, some trends were more than obvious; in the control group almost all cultures showed positive results were as those in Metronidazole & Vitamin E group were most sterile. However the difference between Metronidazole & Vitamin E group, Vitamin E group and Metronidazole group was not as apparent as in other variables.

In our study it was found that on Vitamin E instillation intra-peritoneally, chances of pus pocket formation decrease to 57% the pus over caecum decreased to 42.9% when compared with the control group. It is clear that Vitamin E if instilled intra-peritoneally decreased the lethality of peritonitis. It is a safe and efficient option to use Vitamin E intra-peritoneally, proved its efficacy in sheep model of peritonitis.[14] Not only that, Vitamin E results in positive effects on the level of endotoxemia in experimental peritonitis,[15] while in case of metronidazole, if it used intra-peritoneally along with parenteral antibiotics reduces lethality.[16] It also acts as an anti-inflammatory agent[11,15,17,18].

In present study mortality was markedly low in metronidazole & vitamin E group (only one in subgroup III) than control group (4 in subgroup III, 3 in subgroup II and 2 in subgroup I) while in Vitamin E (3 in subgroup III, 2 in subgroup II and 1 in subgroup I) and Metronidazole (2 in subgroup III, 1 in subgroup II and 1 in subgroup I) it was overlapping. [10,18] It can be assumed that Vitamin E plays an important role in experimental peritonitis especially in reducing the mortality.[10,12,18]

The cause behind all these reactions is bacterial adherence to mucosal surface which increases the bacterial translocation.[19,20] In experimental studies apart from vitamin E other agents like Manitol, Desferrioxamine, Allopurinol were used to prevent the intestine from damage due to oxygen derived free radicals.[21]

We also found that use of vitamin E is beneficial in experimental models; our study is also consistent with these studies[22,23,24]. This study sufficiently demonstrates the superiority of "Metronidazole & Vitamin E" combination over all tested groups[3]; shown that the free radical scavengers when used in combination with antibiotic give good results after perforation peritonitis[3,12] the difference between Metronidazole group and Vitamin E group is too narrow for a definite statement but the trends favor vitamin E over Metronidazole. Even when the results fail to be statistically significant the percentage trend is strikingly constant and sufficiently large enough to expel all doubts paving way for a valid and verifiable conclusion to be drawn.

5. Conclusion

The rat peritonitis model of caecal ligation and puncture is satisfactory and gives good result. The size of perforation is directly related to the severity of sepsis and as the size of perforation increases, the effect of peritonitis increases. Adhesion formation was a constant feature, thus it probably results from peri-operative trauma to the gut and the effect of peritonitis, as a disease is rather contributory. There is no effect on adhesions. The difference between Vitamin E group and Metronidazole group is very little and though Vitamin E scores better on most of the counts, the difference is too narrow for a justifiable definite conclusion to be safely drawn. The antibiotic and antioxidant combination is superior to control group and to the antibiotic or antioxidant group when used alone. However, this combination requires further evaluation specifically in human studies.

References

1. Wang X, Quinn PJ. Vitamin E and its function in membranes. *Prog Lipid Res* 1999; 38(4): 309-336.
2. Chaudiere J, Ferrari-Iliou R. Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem Toxicol*

1999; 37(9-10): 949-962.
3. Castillo M, Toledo-Pereyra LH, Gutierrez R, Prough D, Shapiro E. Peritonitis after cecal perforation. An experimental model to study the therapeutic role of antibiotics associated with allopurinol and catalase. Am Surg 1991;57: 313-316.
4. Zhang H, Slutsky AS, Vincent JL. Oxygen free radicals in ARDS, septic shock and organ dysfunction. *Intensive Care Med* 2000; 26(4): 474-476.
5. Rotstein OD. Oxidants and antioxidant therapy. *Crit Care Clin* 2001; 17 (1): 239-247.
6. Lehmann C, Egerer K, Georgiew A, Weber M, Grune T, Kox WJ. Inhibition of tumor necrosis factor-alpha release in rat experimental endotoxemia by treatment with the 21-aminosteroid U-74389G. *Crit Care Med* 1999; 27(6): 1164-1167.
7. Wichterman KA, Baue AE, and Chaudry AH. A sepsis and septic shock – A review of laboratory models and proposal. *Journal of Surgical Research* 1980; 29: 189-201.
8. Otero-Anton E, Gonzatez-Quintela A, Lopez-Sota A, Lopez-Ben S, Lloro J, Perez LF. Cecal Ligation and Puncture as a model of Sepsis in the rat: Influence of the Punctsure Size On mortality, Bacteremia, Endotoxemia and Tumor Necrosis Factor Alpha levels. *Eur Surg Res* 2001; 33: 37-79.
9. Razdan JL, Jain SP, Razdan S, Husain MT, Srimannarayan A, Burman AK. Post-operative intraabdominal adhesions an experimental study. *Indian Journal of Surgery* 1973; 35: 230-234.
10. Soybir N, Soybir G, Lice H, Dolay K, Ozseker A, and Koksoy F. The effects of desferrioxamine and vitamin E as supplements to antibiotics in the treatment of peritonitis in rats. *Journal of Royal College of Surgeon of Edinburgh*. 2002; 47: 700-704.
11. Konukoglu D, Ercan M, Ziylan E. Trace element levels in the experimental peritonitis. *J Trace Elem Med Biol* 2001; 15: 115-118.
12. Powell RJ, Machiedo GW, Rush BF Jr, Dikdan GS. Effect of oxygen-free radical scavengers on survival in sepsis. *Am Surg.*1991 Feb;57(2):86-8.
13. Demling R, LaLonde C, Ikegami K, Picard L, Nayak U. Alpha-tocopherol attenuates lung edema and lipid peroxidation caused by acute zymosan-induced peritonitis. *Surgery.* 1995 Feb;117(2):226-31.
14. Toutain PL, Hidiroglou M, Charmley E. Pharmacokinetics and tissue uptake of d-alpha-tocopherol in sheep following a single intraperitoneal injection. *J Diary Sci* 1995; 78: 1561-1566.
15. Vlasov AP, Tarasova TV, Sudakova Glu, Ashirov RS, Kil'diushov AN, Dubovskaia TN, Rubtsov OIu, Lazareva OA. Effect of antioxidants on endotoxicosis in experimental peritonitis. *EKSP Klin Farmakol* 2000; 63: 58-61.
16. McAvinchey DJ, McCollum PT, Lynch G. Towards a rational approach to the treatment of peritonitis: An experimental study in rats. *Br J Surg* 1984; 71: 715-717.
17. Kono Y, Inomata M, Hagiwara S, Shiraishi N, Noguchi T, Kitano S. A newly synthetic vitamin E derivative, E-Ant-S-GS, attenuates lung injury caused by cecal ligation and puncture-induced sepsis in rats. *Surgery.* 2012 Mar;151(3):420-6.
18. Jiang Q, Lykkesfeldt J, Shigenaga MK, Shigeno ET, Christen S, Ames BN. Gamma-tocopherol supplementation inhibits protein nitration and ascorbate oxidation in rats with inflammation. *Free Radic Biol Med*. 2002 Dec 1;33(11):1534-42.
19. De la Portilla F, Ynfante I, Bejarano D, Conde J, Fernández A, Ortega JM, Carranza G. Prevention of peritoneal adhesions by intraperitoneal administration of vitamin E: an experimental study in rats. *Dis Colon Rectum*. 2004 Dec;47(12):2157-61.
20. Horgan AF, Stuart EM, O'Shaughnessy B, Cryan B, Kirwan WO. Bacterial translocation during peroperative colonic lavage of obstructed rat colon. *Br J Surg* 1994; 81:1796-8.
21. Katayama M, Xu D, Specian RD, Deitch EA. Role of bacterial adherence and the mucus barrier on bacterial translocation. *Ann Surg* 1997; 225:317-26.
22. Barber AA; Bernheim F. Lipid peroxidation: Its measurement, occurrence, and significance in animal tissues. *Advances Geront Res* 1967; 2:355-60.
23. Reis E, Kama NA, Coskun T, Korkusuz P, Ors U, Aksoy M, et al. Effects of octreotide and a-tocopherol on bacterial translocation in experimental intestinal obstruction: a microbiological, light and electronmicorscopical study. *Hepatogastroenterology* 1997; 44:656-63.
24. Marubayashi S, Dohi K, Sugiro K. The protective effect of administered a -tocopherol against hepatic damage caused by ischemia-reperfusion or endotoxemia. *Ann N Y Acad Sci* 1989; 570:208-18.
25. Sato K, Niki E, Shimasaki H. Free radical mediated chain oxidation of low density lipoprotein and it's synergistic; inhibition by E and C. *Arch Bioch Bioph* 1990; 279:402-5.